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A SIMPLE METHOD OF DEMONSTRATING THE PRODUCTION OF ALDEHYDE BY CHLOROPHYLL AND BY ANILINE DYES IN THE PRESENCE OF SUNLIGHT[1]

W. J. V. Osterhout

In 1906 it was stated by Usher and Priestly[2] that chlorophyll, extracted from leaves and spread out in thin films (under special conditions), can decompose CO_2 and produce formaldehyde in sunlight.

In the following year the writer attempted to repeat their experiments but found the method unsatisfactory; as a result a simpler method was devised which proved so successful that it has been employed by his classes ever since.

The method consists in extracting chlorophyll from fresh leaves by means of alcohol; shaking the alcohol[3] with carbon tetrachloride, drawing off the latter, sprinkling it on filter paper, and allowing it to evaporate. For this purpose the filter paper is stretched in a suitable manner (or merely hung over two parallel glass rods) and the extract sprayed over it until the paper is saturated. In the course of a few minutes, when most of the solvent has evaporated, the paper is again sprinkled with the extract. This is repeated until the paper acquires a deep green color, comparable with that of a leaf.

When the paper is dry it is placed in a bell jar, forming a lining which covers the entire surface: it is then wet with water (which incidentally helps to keep it in position by making it adhere to the glass). The bell jar is then inverted over a glass plate on which is a large petri dish containing about 5 cc. of water. The edges of the bell jar are sealed by means of vaseline. It is desirable to use a large bell jar and to cover the inside completely with filter paper. Several are prepared, some of which are placed in the light while others, kept in the dark, act as controls.

Those which are placed in the light are allowed to remain undisturbed until the chlorophyll has been bleached to a pale green or a pale yellow color.[4] The bell jar is then removed and the water in the dish is tested for aldehyde by means of Schryver's test.[5] A positive result is obtained in the majority of cases.

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[1] An abstract appeared in Science n. ser. **42**: 68. 1915.

[2] Proc. Roy. Soc. Lond. B. **77**: 369. 1906; **78**: 318. 1906.

[3] The addition of water to the alcohol may be necessary to ensure separation.

Evidently a volatile aldehyde is produced which diffuses through the air and becomes absorbed by the water. Care is taken to place the dish so that it does not receive drippings from the filter paper. The experiment succeeds in many cases even without the use of a bell jar if the paper is simply folded into a cone and inverted over a small dish of water.

The controls kept in the dark do not give the test in any instance. It is therefore evident that if the chlorophyll contained aldehyde before exposure to sunlight the amount was too small to give a test.

It seemed desirable to ascertain whether other substances could be substituted for chlorophyll in this experiment. Accordingly a series of aniline dyes were employed. A considerable number gave positive results. The most reliable were methyl green and iodine green. Aqueous solutions were sprayed upon filter paper and allowed to dry. This was repeated until the paper was deep green in color. The paper was then exposed to sunlight in the bell jar for several days in succession until the color had practically disappeared. A positive test for aldehyde was obtained in the majority of cases, while the controls in the dark gave no test.

It therefore appeared as if the production of aldehyde was similar in the case of chlorophyll and aniline dyes, and that if the experiments of Usher and Priestly had any bearing on photosynthesis the experiments on aniline dyes were of considerable interest.

At that time no criticisms of Usher and Priestly's work had appeared, and the writer undertook experiments to test the validity of their conclusions regarding photosynthesis. These experiments showed that it makes practically no difference in the result whether CO_2 is excluded altogether from the bell jar or whether the concentration is increased to 10 percent but it was found that the presence of oxygen is necessary. The writer therefore came to the conclusion that the aldehyde is not produced by the decomposition of CO_2 but rather by the decomposition of chlorophyll.

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[4] This may require several days.

[5] The test is made as follows: Add to 10 cc. of the solution to be tested:

2 cc. of 1 percent phenylhydrazine hydrochloride (freshly prepared and filtered).

1 cc. 5 percent potassium ferricyanide (freshly prepared).

5 cc. concentrated HCl.

On adding a drop of amyl alcohol or chloroform and shaking vigorously, the pinkish color will become concentrated in the chloroform.

The test is given by a variety of aldehydes. *Cf.* Schryver, S. B. Proc. Roy. Soc. Lond. B. **82**: 226. 1910.

Subsequently a number of criticisms of the work of Usher and Priestley have appeared, to which these authors have replied.[6] Recently several authors[7] have stated that the aldehyde produced in Usher and Priestley's experiments is the result of oxidative decomposition of chlorophyll. Wager and Ewart believe that decomposition of chlorophyll, and the resulting formation of aldehyde, is a regular step in photosynthesis. There is some evidence in favor of this view,[8] but at present it cannot be said that there is any convincing proof of its correctness.

SUMMARY

1. A method is described by which aldehyde is obtained from chlorophyll in sunlight; the yield is relatively large and it is free from contamination by non-volatile substances.
2. A similar production of aldehyde is observed when certain aniline dyes are substituted for chlorophyll.

HARVARD UNIVERSITY,
LABORATORY OF PLANT PHYSIOLOGY

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[6] *Cf.* Usher, F. L., and Priestley, J. H. Proc. Roy. Soc. Lond. B. **84**: 111. 1911.

[7] *Cf.* Wager, H. Proc. Roy. Soc. Lond. B. **87**: 386, 596. 1914; Warner, C. H. Ibid. **87**: 378. 1914; Ewart, A. J. Ibid. **89**: 1. 1915; Jorgensen, I., and Kidd, F. Ibid. **89**: 342. 1916. For the decomposition of other substances in light, with production of aldehyde, see Neuberg, C. Biochem. Zeit. **13**: 305. 1908; Spoehr, H. A. Biochem. Zeit. **57**: 95. 1913.

[8] That chlorophyll or some compound of it may decompose during photosynthesis is indicated by a recent study of the dynamics of the process. See Osterhout, W. J. V., and Haas, A. R. C. Proc. Nat. Acad. Sci. **4**: 85. 1918. Journal of General Physiology **1**[1]. 1918.